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Fiber type and metabolic characteristics of lion (*Panthera leo*), caracal (*Caracal caracal*) and human skeletal muscle

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ABSTRACT

Lion (*Panthera leo*) and caracal (*Caracal caracal*) skeletal muscle samples from *Vastus lateralis*, *Longissimus dorsi* and *Gluteus medius* were analyzed for fiber type and citrate synthase (CS), 3-hydroxyacyl Co A dehydrogenase (3HAD), phopshofructokinase (PFK), creatine kinase (CK), phosphorylase (PHOS) and lactate dehydrogenase (LDH) activities and compared to human runners, the latter also serving as validation of methodology. Both felids had predominantly type IIx fibers (range 50 – 80%), whereas human muscle had more type I and IIa. Oxidative capacity of both felids (CS: 5 – 9 µmol/min/g ww and 3HAD: 1.4 – 2.6 µmol/min/g ww) was lower than humans, whereas the glycolytic capacity was elevated. LDH activity of caracal (346 ± 81) was higher than lion (227 ± 62 µmol/min/g ww), with human being the lowest (55 ± 17). CK and PHOS activities were also higher in caracal and lion compared to human, but PFK was lower in both felid species. The current data and past research are illustrated graphically showing a strong

relationship between type II fibers and sprinting ability in various species. These data on caracal and lion muscle confirm their sprinting behavior.

Keywords: Fiber type, myosin heavy chain, enzyme activities, feline

1. INTRODUCTION

The wild felid species are phenomenal athletes. Not only are they renowned for power, jumping and sprinting capabilities, but are masters of stealth and stalking.

Lions, the world's second largest and only social felid, are primarily known for their brute strength. The average weight of an adult male can range between 180 to 225 kg (mean 190 kg), whereas the females average approximately 128 kg (Skinner and Chimimba, 2005). Although not the fastest land animal (reaching speeds of up to 70 km/h during short sprints), lions are known as fierce killers and can easily take down animals their own size. The female lions are the primary hunters whereas the males rarely aid in killing prey, but conserve themselves to defend the pride against other male lions. Lion physiology is designed to allow stalking prey for long periods of time, whereafter a short chase ensues. When hunting as a pride, female lions can take down zebra (± 300 kg), buffalo (± 600 kg), eland (± 1000 kg) and female elephants (± 4000 kg). This characteristic is largely a result of their ability to work together as a group rather than that of individual muscle strength. However, being physically fit is a requirement to stay part of the pride (Skinner and Chimimba, 2005).

Caracals (also known as the "Rooikat"), on the other hand, are solitary predators. The males are larger than the females, and their weight can range from 6 to 19 kg. The caracal is renowned for its jumping capability and can jump up to 3 meters high from a stationary-crouched position to catch their prey. Apart from birds, their prey also include small livestock, antelope and rabbits requiring a short chase (Skinner and Chimimba, 2005). They are believed do reach a maximum speed of up to 100 km/h, but is yet to be confirmed.

Both animals are stalkers of prey, moving light-footedly and with stealth before the ultimate attack, hence requiring muscle fibers (type and metabolism) to optimize this type of movement. Additionally, to achieve such acceleration velocities would require fibers that could generate enormous amounts of force in very little time (e.g. cheetah accelerates from 0 to 100 km/h in less than 3 seconds) (Williams et al., 1997).

Skeletal muscle tissue is a highly complex organ, and apart for being responsible for generating movement, also serves various other functions (e.g. imports glucose to regulate blood glucose concentration, generate heat, produce inflammatory cytokinase, to name but a few). The primary

components of skeletal muscle responsible for generating motion can be subdivided into the contractile proteins and the metabolism that supplies ATP needed for the former. The contractile properties of an individual muscle fiber depend largely on the myosin heavy chain (MHC) isoform it expresses. Three isoforms, namely MHC I, IIa and IIx are commonly expressed in adult mammalian skeletal muscle, including humans (Acevedo and Rivero, 2006; Hyatt et al., 2010; Kohn et al., 2007c; Quiroz-Rothe and Rivero, 2001). Fibers expressing MHC I have a slow contractile speed, whereas those expressing MHC IIx are much faster (Bottinelli, 2001). A third isoform, MHC IIa gives rise to fibers with a contractile speed leaning towards type IIx. The very fast MHC IIb isoform, commonly found in limb muscle from small animals such as rats and mice, are rarely found in the limbs of larger animals, but may be expressed in specialized muscles, such as the larynx or eye (Kohn and Myburgh, 2007; Toniolo et al., 2008).

Type I fibers primarily obtain ATP for contraction from aerobic metabolism, with fat and the oxidation of pyruvate *via* the Kreb's cycle being the primary sources, making these fibers more resistant to fatigue. They also contain large numbers of mitochondria, have a dense capillary supply, small cross-sectional area (CSA) and a high myoglobin concentration, explaining their distinctive red color. Type IIx fibers primarily rely on anaerobic metabolism, which includes ATP replenishment via glycolysis and use of phosphocreatine stores. These fibers have very few mitochondria, low capillary supply, are large in CSA, pale in color and fatigue easily. Type IIa fibers show characteristics of both fatigue resistance, yet fast contraction velocities (Essén-Gustavsson and Henriksson, 1984; Kohn et al., 2007a; Saltin and Gollnick, 1983). These type I, IIa and IIx fibers are also classified as a combination of contractile speed and metabolism and therefore known as slow twitch oxidative, fast twitch oxidative and fast twitch glycolytic, respectively.

Although a few studies have described the skeletal muscle characteristics of domestic cats (Hyatt et al., 2010; Toniolo et al., 2008), very little is known about the wild felid species. Presently, only the cheetah (not a true felid) and tiger have been studied. Both have large proportions of type IIx fibers, with the primary source of ATP generation in cheetah muscle from anaerobic metabolism (Hyatt et al., 2010; Williams et al., 1997).

As no data currently exist on lion and caracal skeletal muscle, the aim of this study was to investigate and describe the skeletal muscle fiber type and metabolic profiles of these two wild felids and to relate these findings to their typical physical activity profiles.

2. METHODS

2.1. Sample collection and storage

Two female lions, 1 female caracal and 9 male human endurance runners where used in this study. The lions and human subjects were part of earlier studies for which ethical approval were

obtained from the Animal Use and Care Committee (University of Pretoria) and the Human Research Ethics Committee (University of Cape Town), respectively. Biopsies from the human runners were donated to this study to additionally aid in validating the techniques and serve as control parameters. They included athletes specializing in various distances from 5 km to half marathon runners. Muscle samples from sprinters were not available. The wild caracal was killed on a game farm by a dog and the material provided to the authors. Human samples were collected by biopsy. The two female lions were classified as healthy, having no visible signs of illness or macroscopic abnormalities on dissection. They were between 2 and 3 years old, weighed ±120 kg each, housed together in a 30 hectare enclosed camp and fed 35 kg fresh meat every 4 to 5 days. The muscle samples were obtained after the animals were euthanized at Onderstepoort Veterinarian Clinic. Samples from all animals were collected within 1 hour of death.

Skeletal muscle samples from the mid portion of the *Vastus lateralis*, *Longissimus dorsi* and *Gluteus medius* were obtained for lion and caracal, whereas biopsies were obtained from the *Vastus lateralis* of human subjects. All muscle samples were divided into smaller pieces (30 – 40 mg wet weight) and rapidly frozen in liquid nitrogen and stored at -87 °C until analyses.

2.2. Homogenate sample preparation for enzyme activities and SDS-PAGE

Samples were prepared as described by Kohn *et al.* (2007c), with modifications. A small piece of frozen tissue was weighed and 100 mM potassium phosphate buffer, pH 7.30, added to a ratio of 1:19. The tissue was homogenized on ice using a Teflon tip, after which it was sonicated twice for 10 seconds at 6 W using a micro sonication probe (Virtis Virsonic Ultrasonic Cell Disrupter 100) and centrifuged at 1700xg for 5 minutes (4 °C). Enzyme assays were performed using the supernatant, whereas a small part of the pellet was diluted with sample buffer (5% β-MEtOH, 2.5% SDS, 10% glycerol, 62.5 mM Tris, pH 6.8 and 0.1% bromophenol blue). These latter samples were heated to 95 °C for 3 minutes and used for determining the MHC isoform content and Western blot analyses. A rat muscle sample obtained from a previous published study were included to serve as control for the MHC IIb isoform on Western blots (Smith et al., 2008).

2.3. Enzyme assays

Enzyme activities that served as markers for the respective metabolic pathways, were chosen as follows: creatine kinase (CK) for rapid ATP replenishment capacity, phosphorylase (PHOS) for glycogen breakdown capacity, phosphofructokinase (PFK) for flux capacity of glycolysis, lactate dehydrogenase (LDH) for lactate production, citrate synthase (CS) for Kreb's cycle oxidative capacity and 3-hydroxyacetyl Co A dehydrogenase (3HAD) for fat oxidation capacity. Enzyme activities were determined spectrophotometrically at 25 °C using methods described by Srere (1969), Chi *et al*. (1983) and Essén-Gustavsson and Henriksson (1983), with modifications. All reagents were from Sigma, the final volume in the cuvette 1000 µl and the absorbance wavelength

340 nm, except for CS, which was 412 nm. For each reaction, the maximum slope was determined and expressed relative to the amount of tissue (µmol/min/g ww).

3HAD: 50 mM Tris, pH 8.00, 4 mM EDTA, 60 µM NADH, 50 µM acetoacetyl Co A, 10 µl sample.

CK: 100 mM imidazole, pH 6.70, 2 mM EDTA, 10 mM Mg-acetate, 4 mM glucose, 2 mM ADP, 5 mM AMP, 30 mM phosphocreatine, 10 µM diadenosine pentaphosphate, 2 mM $NADP^+$, 20 mM n-acetylcysteine, 0.5 U/ml hexokinase (Roche), 0.3 U/ml glucose-6-phosphate dehydrogenase, 3 µl sample.

CS: 100 mM Tris, pH 8.2, 0.1 mM 5,5'-dithiobis-(2-nitrobenzoate) (DTNB), 0.4 mM oxaloacetate, 0.16 mM acetyl Co A, 10 µl sample.

LDH: 50 mM Tris, pH 8.00 and 4 mM EDTA, 0.12 mM NADH, 2 mM pyruvate, 3 µl sample.

PFK: 50 mM Tris, pH 8.00, 2 mM EDTA, 5 mM $MgCl_2$, 0.01% BSA, 0.12 mM NADH, 2 mM fructose-6-phosphate, 0.07% β-mercaptoethanol, 2 mM ATP, 0.45 U/ml aldolase, 5.8 U/ml glyceraldehyde-3-phosphate dehydrogenase, 58 U/ml triosephosphate isomerase, 5 µl sample.

PHOS: 37.6 mM potassium phosphate buffer, pH 7.00, 0.75 mM EDTA, 3.75 mM $MgCl_2$, 1.5 mg/ml glycogen, 0.45 mM $NADP^+$, 37.5 µM glucose-1,6-bisphosphate, 0.75 mM AMP, 3.75 U/ml phosphoglucomutase, 3.75 U/ml glucose-6-phosphate dehydrogenase, 3 µl sample.

2.4. Myosin heavy chain isoform content and Western blot analyses

The relative MHC isoform content was determined using SDS-PAGE as developed by Talmadge and Roy (1993) and adapted by Kohn et al. (2007c). Briefly, a volume of sample was loaded on 7% polyacrylamide gels containing 30% glycerol and run in the cold for 24 hours, first at constant 70 V for 4 hours, followed by 20 hours at 275 V. Gels were subsequently silver stained, scanned and analyzed using the Un-Scan-It (Silk Scientific Corporation) software package. The protocol successfully separated the isoforms into three bands for human and lion skeletal muscle, whereas four bands were separated for that of caracal and rat (rat not shown).

To validate the character of the isoforms, Western blot analyses were performed on the same samples. After electrophoresis, the proteins were transferred to PVDF membranes overnight at 90 mA, washed in Tris buffered saline containing 0.1% Tween 20 (TBS-T) and blocked for 1 hour in 5%-non-fat dried milk in TBS-T. Monoclonal primary antibodies specific to the three MHC isoforms were diluted 1:500 in TBS-T and the membranes rotated overnight in the cold. After washing in TBS-T, the membranes were probed with horseradish peroxidase linked secondary antibodies for

1 hour, washed and chemiluminescence reaction performed (Pierce SuperSignal, Pierce, USA), whereafter the membranes were exposed to X-ray film. These films were scanned as above and analyzed for primary antibody reactivity.

Monoclonal primary antibodies were obtained from the Developmental Studies Hybridoma Bank, Iowa. These were all previously validated for specific isoform reactivity in different species and corresponded to MHC I (BA-D5), MHC IIa (2F7), MHC IIx (6H1) and MHC IIb (BF-F3, 10F5) (Acevedo and Rivero, 2006; Hyatt et al., 2010; Lucas et al., 2000; Schiaffino et al., 1989).

2.5. Histochemical and immunohistochemical analyses

Serial cross sections (10 µm thick) were cut on a cryostat at -25 °C and stained using the NADH-tetrazolium stain to determine oxidative capacity of the muscle (Novikoff et al., 1961). On separate sections, type I and II fibers were visualized using ATPase stability at alkaline pH 10.3 (Brooke and Kaiser, 1970).

Immunohistochemistry was performed by incubating sections with the specific MHC antibodies as described by Smith et al. (2008), but with modifications. Briefly, sections were fixed in pre-chilled methanol for 10 minutes at -20 °C and hydrated in 0.05% Tween 20 phosphate buffered saline, pH 7.40 (PBS-T). Sections were then blocked with 5% bovine serum albumin (BSA) in PBS-T for 1 hour at room temperature in a humidified chamber. Primary antibodies diluted 1:25 in PBS-T were added to the sections and incubated overnight at 4 °C in the dark. The following day, the sections were washed twice in PBS-T for 2 minutes and incubated with horseradish peroxidase conjugated secondary rabbit anti-mouse antibody (1:50 diluted in PBS-T) for 1 hour at room temperature. After washing twice in PBS-T for 5 minutes, the immuno-reactive sites were visualized using the peroxidase DAB substrate kit (DAKO Laboratories, Denmark). After adequate washing, the sections were mounted with DPX (BDH laboratories).

All sections were inspected under a microscope (PrimoStar, Carl Zeiss MicroImaging, Germany) and photographed using a CMOS camera. Fibers were classified as type I, IIa and IIx according to the intensity profile of the ATPase, NADH and immunohistochemical stained sections. Hybrid fibers were disregarded as they represented less than 1% of the total number of fibers analyzed. After fiber identification, CSA (μm^2) of each were determined on the pH 10.3 slides using pre-calibrated software (AxioVision ver 4.81, Carl Zeiss MicroImaging, Germany).

2.6. Statistical analyses

Where applicable, values are expressed as mean ± standard deviation. Due to a small sample size of the feline species, values of muscle samples were pooled to represent an overall view of the

animal. Relationships were tested using the Pearson's correlation coefficient. For the latter, a one-way ANOVA with a Tukey *post hoc* test was used to indicate statistical significance ($P < 0.05$).

3. RESULTS

Due to a small sample size for the two animal species, their data from individual muscle groups (Table 2 and Fig. 1) were averaged in order to obtain an overall representation of the animals, as well as to statistically compare the two felids with that from human muscle.

3.1. Enzyme activities

As a whole, CS and 3HAD activities of lion and caracal muscle were approximately 3.5x and 2.5x lower, respectively, than activities measured in human athletes (Table 1). Surprisingly, PFK activities of both felid species were lower than that of human muscle, and different from each other. On the other hand, LDH activity of lion and caracal was 4x and 6x higher, respectively, than activities measured in human athletes. The pathways representing high-energy phosphates (CK) and glycogen breakdown (PHOS) were approximately 2x higher in the animals compared to humans.

For the felid species only, CS and 3HAD activities of the individual muscle groups were similar (Figs. 1A and 1B). Both PFK and LDH seemed to be unaffected by the muscle groups, but a clear species interaction existed, i.e. caracal had higher enzyme activities in all three muscle groups analyzed compared to the lions (Figs. 1C and 1D). This interaction was not evident for CK and PHOS activities measured in the three muscle groups of the two species (Figs. 1E and 1F).

3.2. MHC isoform identification

Both immunohistochemistry and Western blot analyses were used to identify the MHC isoforms in lion and caracal. Gel electrophoresis separated the MHC isoforms from caracal and lion muscle into four and three bands, respectively (Fig. 3A). The antibody 2F7 only recognized one band in all the species and corresponded to the migratory level of the human MHC IIa band (Fig. 3B). The MHC IIx antibody (6H1) only recognized one band for each of the species (Fig. 3C). However, these bands migrated differently from one another, suggesting a difference in molecular weight. The lion had the smallest MHC IIx isoform, followed by caracal and human. The position of the IIx isoforms were also confirmed when double labeling was performed with the MHC I (BA-D5) antibody (Fig. 3D). For all three species, only one MHC I band was identified and was similar in size at the lower migratory level.

An extra band for caracal was identified between its MHC IIx and MHC I isoforms on the silver stained gel (arrow in Fig. 3A). The BF-F3 antibody, known to react with the rat MHC IIb isoform,

failed to react with this unknown band (Fig. 3E). Similarly, 10F5 also failed to recognize this unknown band (data not shown).

Immunohistochemical staining using the primary antibodies from Western blot, confirmed their specificity to the three isoforms from each species. BA-D5 and 2F7 only reacted with type I and IIa fibers, respectively, whereas the MHC IIx (6H1) antibody reacted with MHC IIx fibers, but also showed slight cross-reactivity with type I fibers, but not with type IIa fibers (Figs. 2B, 2C, 2D and 2E). None of the fibers from either species reacted with the MHC IIb (BF-F3) antibody (data not shown).

3.3. MHC isoform content

Overall, the predominant isoform expressed in the two felid muscle was MHC IIx, whereas MHC I and IIa dominated the human *Vastus lateralis* muscle (Tables 1 and 2). A combination of all three muscles revealed that the lion expressed less MHC IIx, but more MHC IIa than the caracal. This is also evident when looking at the muscles individually.

3.4. Muscle morphology

Both lion and caracal muscle showed poor staining intensity with the NADH-tetrazolium method when compared with their human counterparts (Fig. 2A). Those fibers expressing MHC I and IIa were darker and darkest, respectively, in intensity compared to the IIx fibers. Alkaline incubation at pH 10.3 and immunohistochemistry using the monoclonal MHC antibodies showed that caracal and lion both had more type IIx fibers in their *Vastus lateralis* than that from human (Figs. 2B – E), confirming the results obtained from SDS-PAGE.

The CSA of the *Vastus lateralis* muscles are presented in Table 1. The predominant fiber types from each species had the largest CSA. Lion and caracal type IIx fibers were 1.6x and 2.1x larger, respectively, than the remainder of their fiber types. The human type I and IIa fibers were larger than their IIx fibers.

3.5. Correlations

No relationships were found between fiber type and the enzyme activities in any of the wild animal muscles sampled in this study. For example, Pearson's correlation coefficients for MHC I vs. CS and 3HAD were r = 0.54 and r = 0.34, respectfully, whereas MHC IIx vs. LDH and CK were r = 0.53 and -0.200, respectively, all not significant. Once the data from the humans were included in the analyses, all correlations became significant with most *P* values less than 0.0001. However, the relationships were deemed insignificant, as all these relationships were the result of species clustering.

4. DISCUSSION

The main findings of this study were that lion and caracal muscle as a whole, had high CK, PHOS and LDH activities and low CS and 3HAD activities, with a predominant expression of the MHC IIx isoform in all the felid muscles analyzed when compared to humans, indicating a species that have great sprinting capability. In contrast, the rate limiting enzyme for glycolysis were significantly lower than that from human muscle.

4.1. MHC isoform characteristics

The antibodies used in this investigation were able to recognize the isoforms on histological sections. Their specificity was validated with the pH 10.30 and NADH stains. Only the 6H1 antibody, specific to the MHC IIx isoform, showed slight cross-reactivity with fibers containing only MHC I.

Using SDS-PAGE, three isoforms were separated for all three species. Western blotting confirmed their nature as MHC I, MHC IIa and MHC IIx (Fig. 3). Both MHC I and IIa of the felids migrated similarly on the gel to that of human muscle. However, the MHC IIx differed significantly, even between the two felids. According to the migratory size, the lion expressed the smallest MHC IIx isoform, followed by that of caracal and human (Fig. 3A). A different amino acid sequence, or a lack thereof, may contribute to this size difference. It is unclear what the effect of this size difference might have on the overall contractility of these fibers. Both Toniolo et al. (2007) and Marx et al. (2006) showed that with an increase in body mass, the unloaded shortening velocity (Vo) of each fiber type decreases. It is interesting though that the largest difference in Vo occurred within the type I fibers of the different species, despite their MHC I showing similar migratory profiles. Conversely, the type IIx fibers showed the largest variation in migratory profiles, but the difference in Vo was less than that observed for type I fibers. However, Toniolo et al. (2007) warned that body mass may only partially predict Vo. For example, between man, pig and cow (in order of increasing mass), the Vo of type I and IIa fibers were similar for man and cow. On the other hand, Vo of type I and IIa fibers of pig were lower and higher, respectively, compared to human and cow muscle. This discrepancy points to a more complex system in what determines the contractile properties of the fibers. It would therefore be difficult to predict whether differences in contractile properties of the caracal and lion exist based solely on body mass, and would require further investigation.

A fourth band was identified for caracal and its position was slightly lower than the identified MHC IIx band. It was initially suspected to be the MHC IIb isoform, but the antibodies specific to MHC IIb (BF-F3 and 10F5) failed to recognize this band. The intensities of the MHC I, IIa and the unknown band from the caracal (Fig. 3A) did not differ substantially. It is therefore speculated that if this unknown band could react to the antibody, then enough protein was loaded. Recently it was shown

that cheetah muscle may express a small amount of MHC IIb in the medial *Gastrocnemius* muscle (Hyatt et al., 2010). However, this isoform was absent in the other muscles analyzed and in all the muscles from the tiger that were studied. Although rare in its expression, MHC IIb have been found in limb muscles of Llama, dogs and pigs and this unidentified band may well present with its characteristics (Graziotti et al., 2001; Lefaucheur et al., 2002; Toniolo et al., 2007). This would require future studies using mRNA and single fiber techniques to explain the characteristics of this as yet unidentified band.

The importance of using multiple techniques to identify MHC isoforms for a species not previously investigated was once again highlighted in this study (Acevedo and Rivero, 2006). Three histological techniques and Western blotting were performed. Of these, antibody 6H1, considered to only react with MHC IIx, was shown to cross-react with MHC I in the felid species, but not humans. It was therefore essential to perform additional stains (ATPase and NADH) to also validate the reactivity of the antibodies. Future studies focusing on this kind of MHC identification should therefore always use a combination of techniques.

4.2. Muscle fiber type and size

On gross examination, felid muscle appeared white in color with red pigment primarily absent. Already this indicated that the muscle may contain a large proportion of fast twitch glycolytic fibers. A clear distinction between the human and feline muscle was the predominance of type IIx and low proportions of type I and IIa fibers in the latter, whereas type I and IIa fibers were more predominant in humans. This is consistent with previous studies on felids and human endurance runners (Hyatt et al., 2010; Kohn et al., 2007b). Caracal also had significantly more type IIx fibers in individual muscles compared to the lion (Tables 1 and 2). Similarly, cheetah muscle has a predominance of type IIx fibers, with the *Vastus lateralis* containing up to 76% (Hyatt et al., 2010; Williams et al., 1997). The largest felid species, the tiger, contained a mixture of fiber types, but a high percentage of IIx fibers were still present in the limb muscles (Hyatt et al., 2010). The requirement of some fibers for endurance may be as a result of the larger felids' hunting strategies. Whereas the caracal and cheetah rely on speed of attack, lion (and possible tigers) often require a combination of power and endurance to hold on to larger prey. This form of attack may continue for prolonged periods, hence the requirement also for some type IIa fibers. Other animals (wild and domestic) showing large numbers of MHC IIx fibers include giraffe, reindeer, horse and various African antelope species (Acevedo and Rivero, 2006; Essén-Gustavsson and Rehbinder, 1985; Kohn et al., 2007c; Serrano et al., 2000; Spurway et al., 1996; Stickland, 1979; Toniolo et al., 2008). The predominance of this fast fiber type is comparative with the known ability to run at great speeds.

The CSA of the type I and IIa fibers of caracal and lion were approximately half that of humans (Table 1), whereas the type IIx fibers of caracal were larger than those of human and lion. This data is in agreement with previous studies. The CSA of the three fiber types of caracal and lion are similar in size to that of bovine, cat, dog, cheetah, horse, blue wildebeest, llama, black bear and reindeer (Acevedo and Rivero, 2006; Graziotti et al., 2001; Marx et al., 2006; Pösö et al., 1996; Serrano et al., 2000; Smerdu et al., 2009; Spurway et al., 1996; Toniolo et al., 2008; Williams et al., 1997; Young, 1982). Human and rhinoceros fiber sizes were all larger than the above. Additionally, it also emphasize that CSA is not related to body mass, but may primarily be genetically determined. However, the differences in CSA does not insinuate that caracal and lion fibers, or that from any other species, are weak as a result of the smaller CSA. The amount of power a muscle could generate during contraction is largely dependent on i) the number of fibers recruited, ii) the fiber type proportions and iii) adequate ATP supply to sustain the cross bridge cycle. Additionally, muscle fiber CSA only partly contribute to the force production of a fiber (e.g. strength training may increase fiber size with a concomitant increase in force). One could therefore argue that, due to the smaller fiber size, the muscle could contain more fibers (primarily genetically determined at birth with large variations between species) and thus lead to greater force production as a whole unit. It would therefore be interesting to isolate measure whole muscle contractility in different species and determine fiber numbers and relate it to the force generating capabilities of their single fibers.

4.3. Muscle metabolism

Overall, the oxidative and fat utilization capacity of lion and caracal muscles from all three muscle groups were substantially lower in comparison with those of humans (Table 1 and Figs. 1A, B and 2A). This indicates that the two felid species have a low endurance capability and agrees with previous studies on human sprinters and cheetah (Dawson et al., 1998; Williams et al., 1997). Specifically, single fiber studies indicated that type IIx fibers have low oxidative capacity compared to type I and IIa fibers and therefore primarily rely on high energy phosphates and anaerobic metabolism of glycogen and blood glucose for ATP replenishment (Essén-Gustavsson and Henriksson, 1984). Indeed, the enzyme markers involved in anaerobic metabolism of these fuels (CK, LDH and PHOS) in the felids were all significantly higher than human, yet lower than cheetah (LDH only), thus agreeing with their preferred activity and hunting strategy. However, an unexpected finding was the low PFK activity in both felids. Being a major rate-limiting enzyme of glycolysis and that these animals probably would rely on anaerobic metabolism due to their nature of being fast sprinters, the opposite was expected. Although low activities of PFK were found in bovine and Rhesus monkeys, comparisons of PFK activities to other fast sprinting species are unfortunately limited and would have revealed more insight into their metabolism (e.g. no PFK activity was measured for cheetahs) (Grichko et al., 1999; Jurie et al., 2006; Williams et al., 1997). This would emphasize the need for more research on the metabolic pathways of these species.

Nevertheless, the high CK activities of the lion and caracal would be able to rapidly supply ATP for short periods of sprinting.

4.4. Comparing the literature

In order to understand the contribution of muscle fiber type to maximum reachable speed, XY plots were constructed and plotted against the fiber type data of lions, caracal and other large mammalian species from past research (Acevedo and Rivero, 2006; Andersen et al., 1994; D'Antona et al., 2006; Dawson et al., 1998; Essén-Gustavsson and Rehbinder, 1985; Harber et al., 2002; Hyatt et al., 2010; Inbar et al., 1981; Karlström et al., 1994; Kim et al., 2005; Kohn et al., 2010; Kohn et al., 2007a; Korhonen et al., 2006; Pösö et al., 1996; Rivero et al., 2007; Serrano et al., 2000; Smerdu et al., 2009; Toniolo et al., 2008; Williams et al., 1997). Fiber type profiles of the hind limb for each species were calculated from the available large muscle groups analyzed (*e.g*. the *Vastus lateralis, Semimembranosus, Gluteus medius, Rectus femoris, Plantaris and Gastrocnemius*) and plotted against the reported maximal speed that these animals and humans may achieve (Figs. 4A – C). It needs to be emphasized that the speeds reported in the figures are values obtained from non-scientific references and requires further investigation. Nevertheless, strong relationships were observed with the muscle fiber types and maximum speed. Type I and IIa fibers correlated negatively with speed (Fig. 4A: $r = -0.73$, $P < 0.01$ and 4B: $r = -0.83$, $P < 0.01$), whereas the type IIx fiber proportions correlated positively with speed (Fig. 4C: $r = 0.85$, $P < 0.001$). This is in agreement with previous research on human distance runners and sprinters, where 40 m sprinting speed correlated positively with the percentage fast twitch fibers (type IIa + IIx) (Inbar et al., 1981). The human sub-groups had few type IIx fibers and were the slowest of all the species included in the figures (Fig. 4C) The cheetah, caracal and lion all had the highest proportions of type IIx fibers, and hence confirm their ability to run at great speeds. From a muscle perspective, the current analyses would therefore suggest that type IIx fibers are the sole determinant of maximal sprinting capability over short distances in these species. On the other hand, with their low type IIx proportions, it is tempting to suggest that humans are more suited for endurance activities. Furthermore, it also suggests that a large genetic component (which may be linked to body mass) is responsible for determining the overall muscle fiber type. However, it is well known that various factors, which include body mass, biomechanics and limb length, can contribute to the overall performance capability of an individual species.

4.5. Limitations and future directions

The primary limitation of the present study was a small sample size that would influence statistical power. Furthermore, no male felids were available for study and their inclusion may have yielded different results. However, these animals are exotic species and currently threatened, and due to strict legislation, samples are difficult to acquire. Secondly, it is well known that muscle fiber type and metabolism differ substantially from superficial to deep regions within the same muscle (Hyatt

et al., 2010; Kohn and Myburgh, 2007). An attempt was made to keep the sampling site and depth constant throughout this study to resemble the same approximate depth obtained for human sampling. Unfortunately, only one location could be sampled for all three species (central-mid portion of each muscle). Future studies should therefore include different depths in order to obtain a more thorough representation of the muscle.

5. CONCLUSION

In conclusion, this is the first study to investigate the fiber type and metabolic profile of lion and caracal skeletal muscle. Both felids had an abundance of type IIx fibers in their *Vastus lateralis*, *Gluteus medius* and *Longissimus dorsi* muscles with ATP supply primarily originating from anaerobic metabolism. These findings agree with their known physical abilities as fast sprinters and jumpers.

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8. TABLES

Table 1 – Enzyme activities and MHC isoform composition of caracal and lion muscles grouped. Human muscle composed only of data obtained from the *Vastus lateralis*. CSA were only determined in the *Vastus lateralis* muscles of the three species. Values are means ± SD. * Different from human ($P < 0.05$), ** Different from human and caracal, † Different from caracal ($P < 0.05$).

Species	Human	Caracal	Lion
Enzymes		µmol/min/g ww	
CS	23.1 ± 4.2	6.2 ± 1.5*	6.7 ± 1.3*
3HAD	5.2 ± 2.1	1.7 ± 0.3*	2.1 ± 0.5*
PFK	27 ± 4	15 ± 3*	6 ± 3**
LDH	55 ± 17	346 ± 81*	227 ± 62**
CK	146 ± 35	274 ± 110*	281 ± 64*
PHOS	23 ± 6	39 ± 8*	45 ± 12*
MHC isoform content		Percentage (%)	
MHC I	43 ± 9	12 ± 6*	16 ± 4*
MHC IIa	43 ± 5	16 ± 2*	27 ± 6**
MHC IIx	14 ± 8	72 ± 6*	57 ± 7**
Cross-sectional area		μm^2	
Type I	5409 ± 1252	2053 ± 698*	2014 ± 432*
Type IIa	5174 ± 1299	1859 ± 587*	2005 ± 475*
Type IIx	2968 ± 554	4235 ± 1119*	3202 ± 657†

Table 2 – MHC isoform composition (%) of the three caracal and lion skeletal muscle groups. Human values are expressed as mean ± SD.

Species	MHC I	MHC IIa	MHC IIx
		Vastus lateralis	
Caracal	18	15	68
Lion 1	22	28	50
Lion 2	12	28	60
Human	43 ± 9	43 ± 5	14 ± 8
		Longissimus dorsi	
Caracal	6	15	79
Lion 1	17	33	50
Lion 2	11	32	58
		Gluteus medius	
Caracal	12	18	71
Lion 1	16	24	60
Lion 2	16	17	67

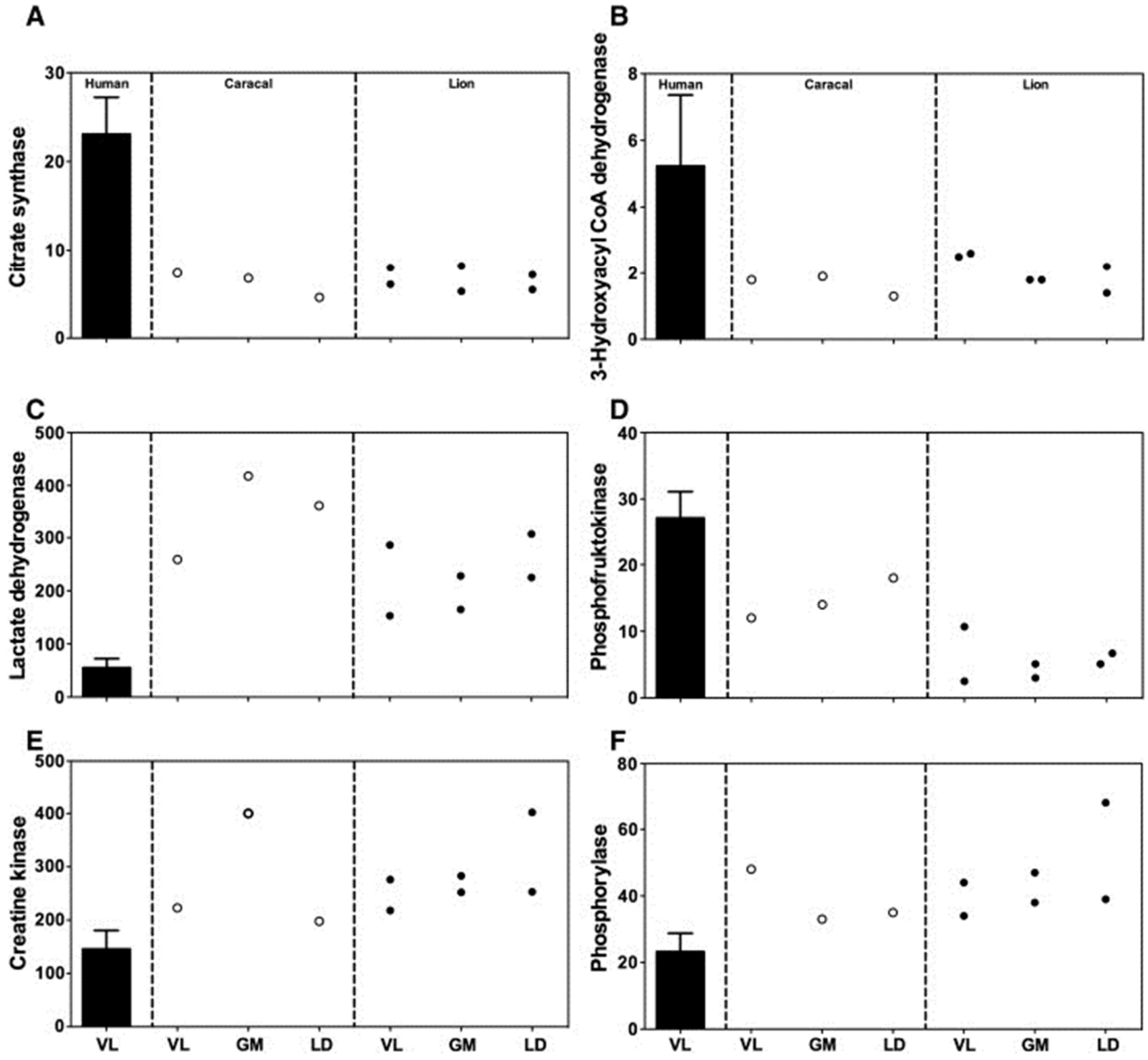

Fig. 1. Enzyme activities (µmol/min/g ww) in *Vastus lateralis* (VL), *Gluteus medius* (GM) and *Longissimus dorsi* (LD) muscles of caracal and lion (circles). The bars represent the mean ± SD of human VL for comparative purposes. A. Citrate synthase. B. 3-Hydroxyacetyl Co A dehydrogenase. C. Phosphofructokinase. D.Lactate dehydrogenase. E. Creatine kinase. F. Phosphorylase.

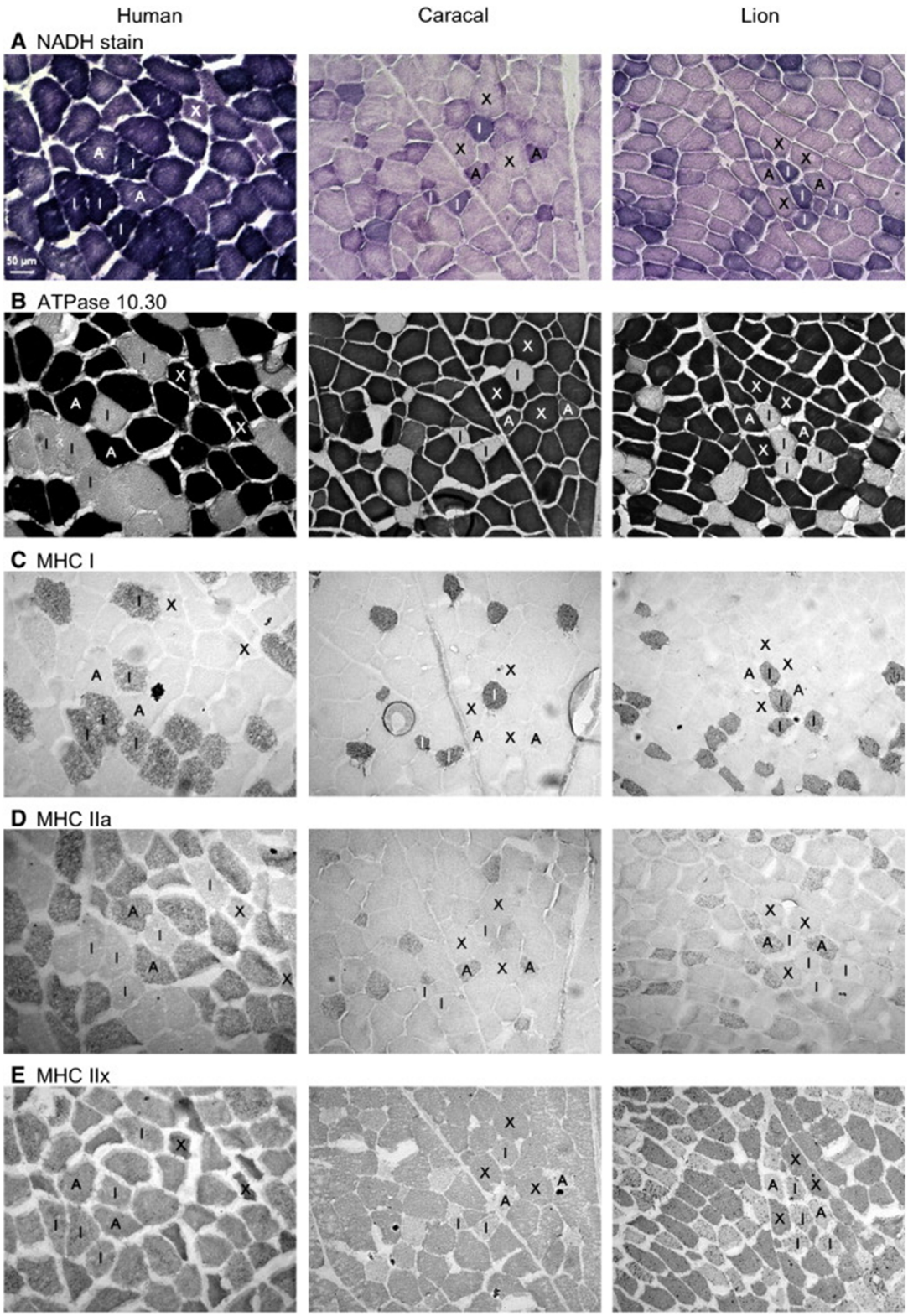

Fig. 2. Histology and immunohistochemistry of skeletal muscle samples from human, caracal and lion. Fiber types: I, type I; A, type IIa; and X, type IIx. A. NADH stain — representing oxidative capacity of fibers.. B. ATPase stain pH 10.3 — fibers not stained equate to type I fibers. C. MHC I (antibody BA-D5) — indicating type I fibers in all three species.. D. MHC IIa (antibody 2F7) — indicating type IIa fibers in all three species.. E. MHC IIx (antibody 6H1) — indicating type IIx fibers in all three species (slight cross-reactivity with lion and caracal type I fibers, but not with type IIa).

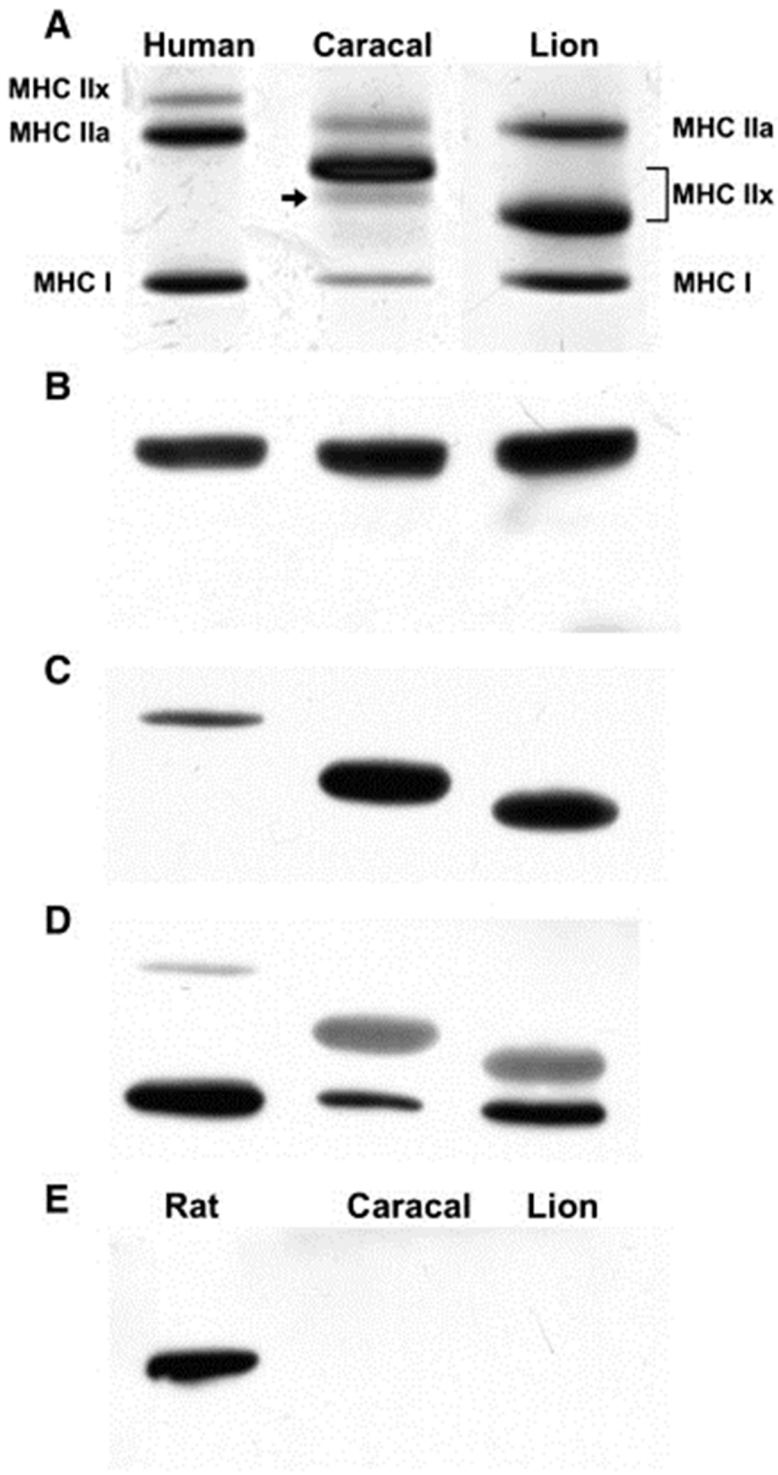

Fig. 3. Myosin heavy chain (MHC) isoform separation and Western blots of human, caracal and lion skeletal muscles. A rat sample was included in 3E as a positive control for MHC IIb. A. Silver stain (arrow indicates band that was not recognizable by any of the primary antibodies used). B. MHC IIa (antibody 2F7). C. MHC IIx (antibody 6H1). D. MHC I and MHC IIx (antibodies BA–D5 and 6H1). E. MHC IIb (antibody BF–F3).

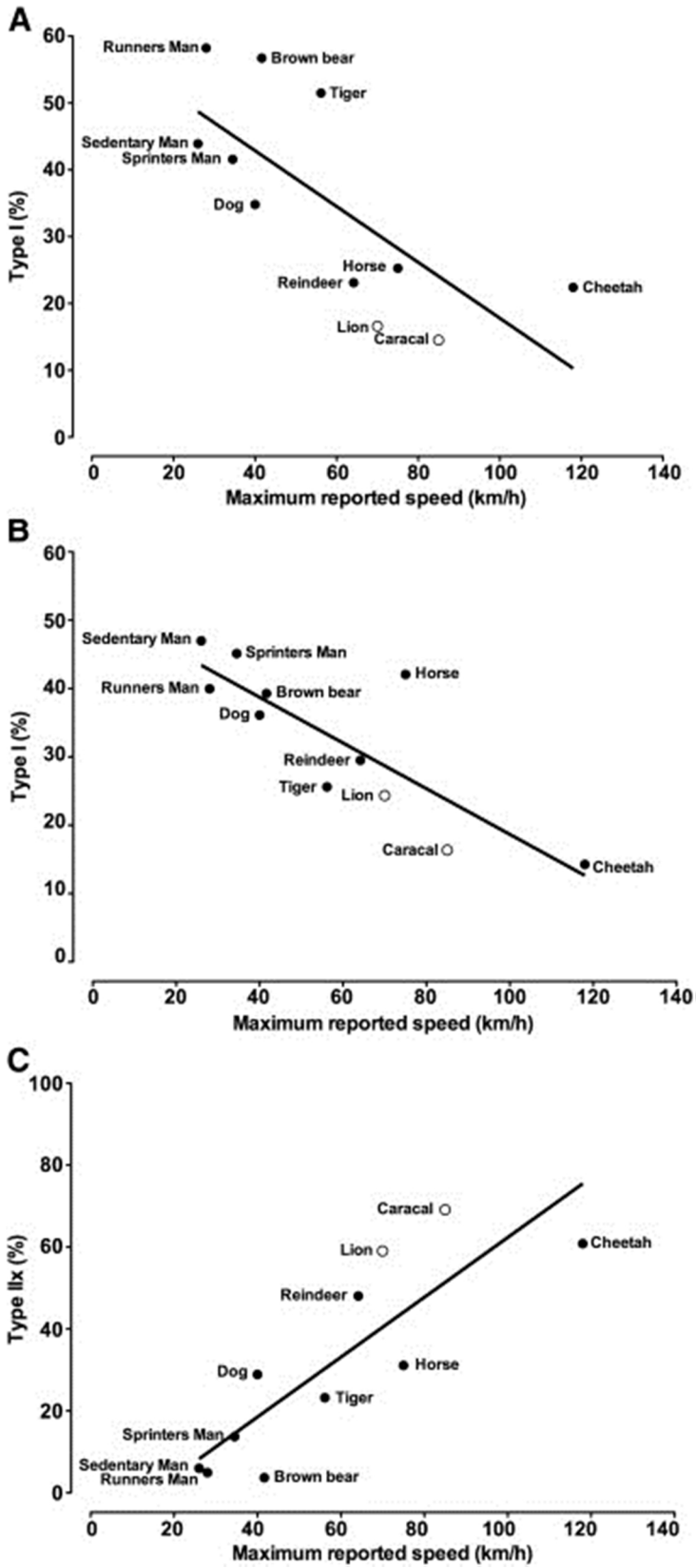

Fig. 4. Relationships between muscle fiber type and maximal sprinting speeds of different mammalian species. Data were obtained from various literature sources (see text for references). Open circles represent species data from the present study. A. Type I fibers ($r = -0.73$, $P < 0.01$). B. Type IIa fibers ($r = -0.83$, $P < 0.01$). C. Type IIx fibers ($r = 0.85$, $P < 0.001$).